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DYNAMICS OF CARBON AND NITROGEN MINERALIZATION, MICROBIAL BIOMASS, AND NEMATODE ABUNDANCE WITHIN AND OUTSIDE THE BURROW WALLS OF ANECIC EARTHWORMS (*LUMBRICUS TERRESTRIS*)

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We conducted a laboratory study using soil cores to determine whether anecic earthworm (*Lumbricus terrestris*) burrow linings (the drilosphere) are sites for enhanced carbon and nitrogen mineralization and increased microbial biomass and nematode abundance. We compared microbial biomass C, C mineralization rates, metabolic quotient, levels of inorganic N (NO_3^- and NH_4^+), and nematode abundance over the course of 11 weeks in soil from earthworm burrows, bulk soil away from burrows, and a control soil in cores to which no earthworms were added. Significant differences were observed in microbial biomass carbon, which was 38 to 84% lower, and carbon mineralization and metabolic quotient, which were 2.3 to 7.5 and 5.6 to 17.4 times, respectively, higher in burrow than in control soil. No significant differences were observed in these variables between bulk and control soil. In addition, nematodes were 3.7 to 6.5 times more abundant, and inorganic N levels 21 to 78% higher in burrow than in control soil, with no significant differences observed between bulk and control soil. Dynamics of microbial biomass carbon and inorganic N followed the same general pattern in burrow, bulk, and control soil. By contrast, dynamics of nematode abundance, carbon mineralization, and metabolic quotient differed between burrow and both bulk and control soil, with peak values observed at 5, 7, and 11 weeks for nematode abundance, C mineralization, and metabolic quotient, respectively. Our results suggest that earthworms may have an indirect effect on soil C and N dynamics by stimulating the activities of nematodes and their interaction with microbial biomass in the drilosphere to a greater degree than is observed in soil that has not come in direct contact with earthworms.

EARTHWORMS increase the rate of nutrient release from bulk soils (Marinissen and de Ruiter 1993) and affect the growth rates of plants in both agricultural and forested ecosystems (Haimi et al. 1992; Wolters and Joergensen 1992). Earthworms may contribute directly to nutrient mineralization through respiration and digestion (Andrén et al. 1990) and mixing litter with mineral soil (Cheshire and Griffiths 1989), or indirectly by controlling the growth rate of microorganisms (Marinissen and de Ruiter 1993). The stimulation of microbial activity by earthworms has been attributed to compensatory microbial growth resulting from earthworms grazing on microflora (Marinissen and de Ruiter 1993) although mucus excretions (Scheu 1991) and aeration of burrows may also stimulate microbial activity. Direct mechanisms are generally regarded as minor (Haimi et al. 1992), whereas indirect mechanisms are believed to be the major component of the effect of earthworms on mineralization (Marinissen and de Ruiter 1993).

Earthworm burrow walls, known as the drilosphere, have physical and chemical properties that are distinct from those of the bulk soil and alter the manner in which water, solutes, and biota interact with burrow soil. Burrow linings have been found to have a narrower range of pH and clay content than bulk soil, with levels of to-

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tal and water soluble carbon two to eight times higher in burrow walls than in the adjacent bulk soil (Stehouwer et al. 1993). Earthworms may affect nutrient mineralization through the creation of moist, nutrient-rich microhabitats for smaller microbivorous organisms, such as nematodes, which in turn stimulate microbial processes, enhancing mineralization.

A laboratory study was conducted to determine whether carbon and/or nitrogen mineralization is enhanced in earthworm burrows and whether the burrows are sites of increased nematode and microbial biomass density. The study was conducted using anecic earthworms (*Lumbricus terrestris*) and packed, forest soil cores under controlled temperature and moisture conditions. We hypothesized that C and N mineralization would be greater in burrow linings than in soil not affected by earthworm burrowing and that nematode numbers and microbial biomass would be higher in burrow soil.

MATERIALS AND METHODS

Experimental Design

Forest soil (Ap and C horizon) was collected in January 1996 from a maple-oak forest on a Hinkley sandy loam soil (sandy-skeletal, mixed, mesic, Typic Udorthent) at the Peckham Farm Research Facility of the University of Rhode Island, Kingston, RI. Ap and C horizon soils were mixed (1:1, vol/vol) in the laboratory, stored in the dark at 4°C for 3 weeks, and subsequently equilibrated at 21–23°C for 1 week before the beginning of the experiment. Mixing the soils was necessary to ensure the physical stability of soil cores during the experiment. The soil mixture had an organic matter content of 7.8%, as determined by loss-on-ignition at 550°C for 4 h. Thirty cores (30 cm long, 15 cm diameter) were prepared by packing the soil mixture into PVC cores to a bulk density of 1.1 g/cm^3. A 5-cm layer of oak-maple litter collected from the same area of the forest from which soil was obtained was placed on top of the soil in all 30 cores. Two adult earthworms (*Lumbricus terrestris*) were placed in each of 15 cores, with the other 15 cores serving as controls. Earthworm density was approximately 110 worms/m^2, within the range observed for temperate forest soils (Edwards and Bohlen 1996). The cores were placed on plastic saucers and incubated in the dark at 18–20°C. The cores received about 500 mL of water per week: half was applied to the bottom by filling the saucer, with other half applied to the top of each core. The mass water content of the soil (determined gravimetrically at 105°C) at sampling time ranged from 0.27 to 0.38 g per g soil over the course of the experiment, corresponding to 60% and 80%, respectively, of the water-holding capacity of the soil.

Sampling

The soil mixture was analyzed (in triplicate) for nematode abundance, soil respiration, soil moisture, microbial biomass C, and inorganic nitrogen (NO_3^--N and NH_4^+-N) levels at the onset of the experiment. Three earthworm and three control cores were sampled destructively after incubation for 3, 5, 7, 9, and 11 weeks. The number of worms and burrows in each core was recorded before sampling. In order to get values representative of the whole core, soil samples were obtained at depths of 0–5 cm, 5–15 cm, and 15–25 cm. In cores containing earthworms, soil was taken from the burrow walls, within 0.5 cm of the inside of the wall (burrow soil), and from at least 2 cm away from the burrow walls (bulk soil). A total of three samples per core (one from each depth) were analyzed for soil moisture, C mineralization, biomass C, and inorganic nitrogen. Soil samples from different burrows within a particular core and depth were pooled for analysis. The quantity of burrow soil was limited, and the amount of soil required for nematode abundance determinations was large (20–25 g). Thus, for nematode analysis, an equal amount of soil (burrow, bulk, or control) from each depth within a core was pooled for determination of nematode abundance.

Inorganic Nitrogen

Inorganic N (NH_4^+ and NO_3^-) levels in soil were determined by extraction of 1 g (wet weight) of soil with 10 mL of 2*M* KCl solution, filtering (Keeney and Nelson 1982), and colorimetric analysis of the filtrate using an automated nutrient analyzer (model RFA 300, Alpkem).

Carbon Mineralization Rate

To determine C mineralization rates, a known amount of soil (1 g wet wt) was placed into a 20-mL glass serum vial, the vial stoppered with a rubber septum and crimped with an aluminum collar. The sealed vials were incubated for 7 days in the environmental chamber in which the cores were stored. We have shown previously that incubation of soil samples under these conditions does not result in a significant decline of oxygen levels in the headspace of the vials (Gorres et al.

1997). At the end of the incubation period a 1.0-mL sample of the gas in the headspace of the vial was removed by displacement using an automated headspace sampler (model 7000, Tekmar). The concentration of CO_2 in the headspace gas sample was measured with a gas chromatograph (model 14A, Shimadzu) fitted with a Porapak Q column (80/100 mesh, 10 ft). Carbon dioxide was converted to methane using a heated (400°C) Ni catalyst and an H_2 gas stream, and the resulting methane was measured with a flame ionization detector. Injector, column, and detector temperatures were 150°, 60°, and 300°C, respectively. Peak areas for CO_2 were determined by electronic integration. Conversion of peak area to mass of carbon dioxide was made by comparison with vials containing a known concentration of CO_2.

Microbial Biomass Carbon

Microbial biomass carbon was determined using the fumigation-incubation method (Jenkinson and Powlson 1976) using 1-g (wet wt.) soil samples in 20-mL glass serum vials. Following evacuation to remove chloroform residues, the vials were sealed and incubated at 25°C in the dark for 10 days. At the end of the incubation period (0–10 days), the concentration of CO_2 in the headspace of the vial was measured as described above. The air in the headspace of the vials was subsequently evacuated and replaced with fresh air five times before incubation resumed for another 10 days. The concentration of CO_2 in the headspace of the vial was measured again at the end of the second incubation period (10–20 days). The CO_2-C produced during the second incubation period (10–20 days) was subtracted from that produced during the initial incubation period (0–10 days), and the difference was used to calculate microbial biomass C using a k_{EC} value of 0.40 (Sparling and West 1988).

Nematode Enumeration

Nematodes were extracted from the soil using the sugar flotation method (Ingham 1994). The extracted nematodes were stored at 4°C in the dark and counted using a dissection microscope within 1 week of extraction. No attempt was made to classify nematodes into trophic groups.

Statistical Analysis

Statistical comparisons with the control soil were made using a one-way analysis of variance. Means separation was accomplished using the Bonferroni *t* test. All statistical tests were evaluated at the 95% confidence level.

RESULTS

Earthworms survived well during the first 3 weeks of the study, with all of the added worms (six) recovered alive from the cores after incubation for 3 weeks (first sampling date). Four worms were recovered from all three cores after incubation for 5, 7, and 9 weeks, and after 11 weeks only one adult worm (presumably a worm added initially) and one juvenile were recovered. Two earthworm burrows per core were present at 3 and 5 weeks, after which four or more burrows were found per core, indicating greater worm activity.

The soil had a relatively low initial microbial biomass C content, undoubtedly the result of mixing soil from Ap and C horizon (Fig. 1). Microbial biomass C declined with time in all treatments throughout the experiment. Significantly lower microbial biomass C was found in burrow than in control soil on weeks 5, 7, 9, and 11. The lowest biomass levels were observed on week 11, and these were 84% of control values. No significant differences were observed between bulk and control soil on any sampling date.

The rate of carbon mineralization in burrow soil peaked after incubation for 7 weeks (Fig. 1) and was 7.5 times higher than in the control soil, whereas carbon mineralization rates in bulk and control soil were constant for the duration of the experiment. Rates were significantly higher in burrow than in control soil at 3, 5, 7, and 9 weeks. Significant differences in C mineralization rate were observed between bulk and control soil only on week 3. The microbial metabolic quotient (qCO_2, i.e., the rate of CO_2 evolved per unit microbial biomass C) in both bulk and control soils was constant for the first 7 weeks, increasing for the subsequent 4 weeks (Fig. 1). By contrast, qCO_2 in burrow soil appeared to increase disproportionally with time, with a 7-fold increase after 5 weeks and a 100-fold increase after 11 weeks. At week 11, metabolic quotient was more than 17 times higher in burrow than in control soil.

Nematode abundance in burrow soil peaked after 5 weeks and was significantly higher in burrow than in control soil throughout the experiment (Fig. 1), with values in burrow soil 3.7 to 6.5 times higher than in control soil. Nematode abundance in bulk and control soil remained relatively constant during the experiment, with no significant differences observed between these

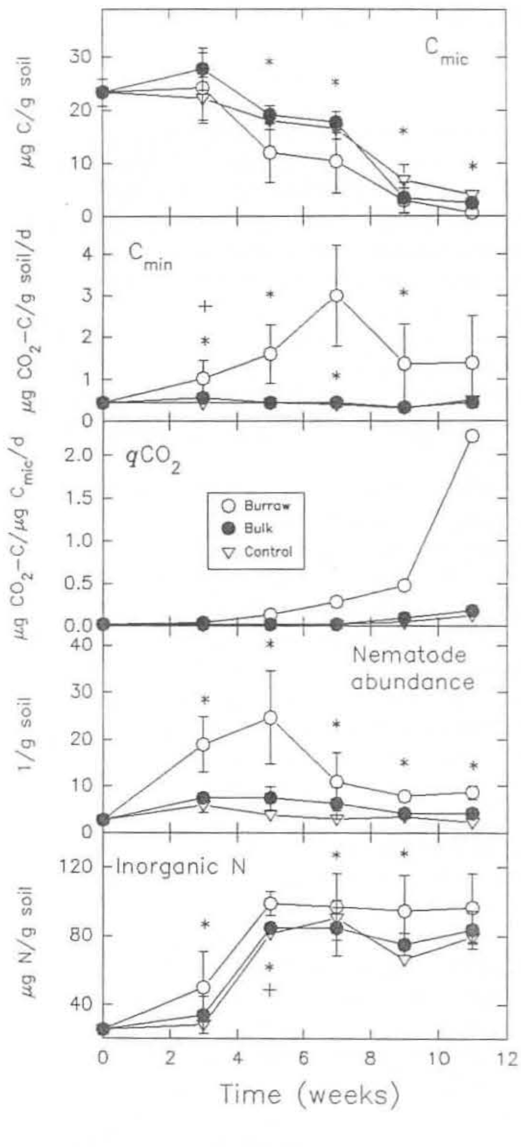

Fig. 1. Microbial biomass carbon (C_{mic}), carbon mineralization rate (C_{min}), metabolic quotient (qCO_2), nematode abundance, and inorganic nitrogen ($NO_3^- + NH_4^+$) levels in burrow, bulk, and control soil as a function of time. (*) indicates significant difference between burrow and control soil; (+) indicates significant difference between bulk and control soil.

two treatments on any sampling date. Significantly lower microbial biomass values co-occurred with significantly higher nematode abundance in burrow soil relative to the control soil in weeks 5, 7, 9, and 11.

Levels of inorganic N ($NO_3^- + NH_4^+$) generally increased for the first 5 weeks, remaining relatively constant for the remainder of the experiment for all treatments (Fig. 1). Burrow soil had significantly higher levels of inorganic N than control soil on weeks 3, 5, 7, and 9. Values for inorganic N were 21 to 90% higher in burrow than in control soil. Significant differences in inorganic N levels between bulk and control soil were observed only on week 5. The mean ($\pm$S.D.) percentage of inorganic N accounted for by ammonium was 13.6 ± 5.0, 9.0 ± 4.2, and 7.0 ± 3.5 for burrow, bulk, and control soil, respectively.

DISCUSSION

The presence of earthworms in soil has been shown previously to reduce microbial biomass C and increase C mineralization as well as levels of inorganic N in soil (e.g., Wolters and Joergensen 1992; Ruz-Jerez et al. 1992; Bohlen and Edwards 1995; Devliegher and Verstraete 1996; Zhang and Hendrix 1995). Our results are generally in good agreement with previous studies. These studies, however, did not differentiate between burrow and bulk soil. The results of the present study indicate that the effects of *L. terrestris* on microbial biomass and C mineralization are restricted to the drilosphere. Others (e.g., Stehouwer et al. 1993) have shown that the drilosphere differs in pH, water soluble organic carbon, and clay content from adjacent, bulk soil. The differences in dynamics of C and N mineralization and fauna provide further evidence of the unique characteristics of soil that has come in contact with earthworms.

It is worth noting that at the earthworm density used in this study (110 individuals per m^2), evaluation of the effects of earthworms on the entire soil (i.e, bulk + burrow soil) would not have revealed any significant differences from the control soil. We evaluated this by combining soil property values for the sampling time on which the greatest differences in nematode abundance between control and earthworm treatments were observed. Values measured for the bulk, x_b, and the drilosphere soil, x_d, were weighed based on the cross-sectional areas of the bulk and burrow soil using the formula

$$x = \pi(R^2-r^2)Nx_d+(1-\pi N(R^2-r^2))x_b \qquad (1)$$

where x is the average property (or variance) for the entire soil, N is the number of earthworms per unit area, r is the radius of burrow, and R is the distance from the center of the burrow to the edge of the visually affected area (soil clearly darkened), with values of r and R of approximately 0.3 and 0.9 cm, respectively. Mean values for all of the soil

variables calculated in this manner from earthworm core data are not significantly different from control cores ($P > 0.1$ in all cases). This analysis stresses further the localized effect of earthworms on soil biota and nutrient cycling.

The effects of earthworms on soil C and N mineralization are generally explained within the context of the "grazing" hypothesis, which states that grazing of soil microorganisms by earthworms stimulates compensatory growth, resulting in an increased metabolic quotient (e.g., Bohlen and Edwards 1995; Wolters and Joergensen 1992; Marinissen and de Ruiter 1993). Our results suggest that an alternative version of the grazing hypothesis may be worth considering: that the creation of burrows by anecic earthworms results in favorable conditions for nematodes, and these members of the soil fauna are at least partially responsible for grazing on microbial biomass and resulting increases in metabolic quotient and inorganic N release observed in the drilosphere.

Involvement of nematodes is suggested by dynamics of nematode abundance, microbial biomass, and C and N mineralization observed in the present study. Increases in nematode abundance in burrow soil during the initial part of the experiment may be the result of two processes: movement and/or reproduction. Nematode movement and aggregation is driven by CO_2 gradients in the soil (Dusenbury 1983, 1987) and bacterivorous nematodes have been shown by Griffiths and Caul (1993) to migrate to decomposing plant residues. Enhanced C mineralization levels observed in burrow soil in week 3 of the study could have attracted nematodes. Alternatively, the presence of litter (anecic earthworms line their burrows with plant materials brought from the surface) and earthworms (dead or alive) and associated exudates could have resulted in increased reproduction of the nematode population in burrow soil. In either case, increases in nematode abundance require food resources, which could have been provided by microbial biomass. The greater rate of microbial biomass loss in the drilosphere and the concurrent enhancement in C mineralization when nematode abundance was increasing is consistent with the hypothesis that nematodes are grazing the microbial community. The metabolic quotient of drilosphere soil increased dramatically subsequent to peaks in nematode abundance and C mineralization. Such increases in metabolic quotient have been interpreted as reflecting the diversion of energy from growth and reproduction to maintenance as a result of disturbance (e.g., Anderson and Domsch 1993).

Data on inorganic N dynamics may also be interpreted as supporting this alternative hypothesis. Grazing of microorganisms by microbivorous nematodes in soil has been shown to increase the release of inorganic forms of N in soil (e.g., Anderson et al. 1981; Ingham et al. 1985) although excretion of inorganic N by earthworms cannot be ruled out as a cause for enhanced levels of inorganic N in burrow soil. In the present study, the enhanced release of inorganic N was observed during the initial stages of the experiment when nematode grazing was presumably decimating the microbial community. Taken together, our results suggest that nematodes grazing on microbial populations in the drilosphere could be partially controlling the dynamics of microbial biomass C and C and N mineralization.

Although the evidence presented here suggests indirect effects of earthworms on C and N dynamics by altering the distribution of nematodes and their grazing activities in soil, our results are not conclusive regarding the involvement of nematodes as grazers. For example, although significant increases in total numbers of nematodes were observed as microbial biomass decreased, a more conclusive case could be made if data on trophic group distribution of nematode populations had been performed, showing a concomitant increase in populations of microbivorous nematodes with declining microbial biomass. Senapati (1992) has shown that the presence the earthworm *Lampito mauritii* (Kinberg) enhances the abundance of microbivorous nematodes in soil. Additional organisms may be involved in grazing along with the nematodes. For example, numerous studies have shown that grazing of bacterial populations by protozoa in soil can have effects on microbial biomass and C and N mineralization similar to those observed in the present study (e.g., Wright et al. 1995; Woods et al. 1982; Rutherford and Juma 1992; Ingham et al. 1985).

In conclusion, our results indicate that nematodes may have an important role as indirect mediators of the effects of earthworms on C and N mineralization in soil.

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